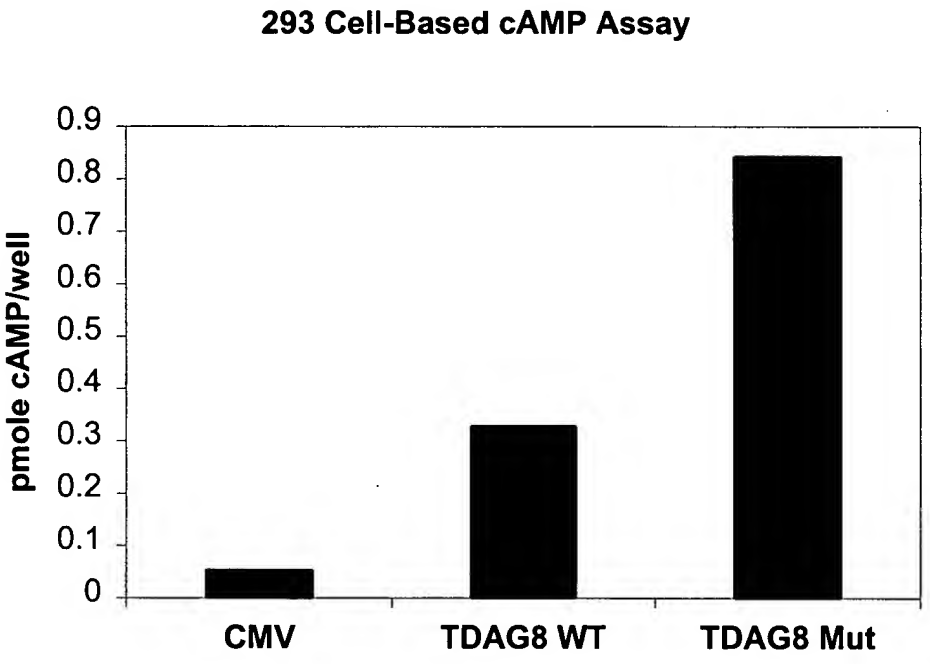
tracer cAMP was added to each well. The plate was placed on a shaker and incubated at room temperature for two hours. Finally, the solution from the wells of the plate were aspirated and the Flashplate™ was counted using a Wallac MicroBeta™ scintillation counter. Data are summarized below, where "CMV" is the control vector; "TDAG8 WT" is the endogenous TDAG8 receptor; and "TDAG8 MUT" is the non-endogenous, constitutively activated version of the TDAG8 receptor:



B. GPR35

A pE2F-Luc Reporter (a component of Mercury Luciferase System 3, Clontech Catalogue # K2053-1) was utilized in 293A cells. Cells were transfected with the plasmid components of this system and the indicated expression plasmid encoding endogenous or non-endogenous GPR35 receptor using Lipofectamine Reagent (Gibco/BRL, Catalogue #18324-012) according to the manufacturer's instructions.

Appendix E

Briefly, 400 ng pE2F-Luc, 80 ng CMV (comprising the GPR35 receptor) and 20 ng CMV-SEAP (secreted alkaline phosphatase expression plasmid; alkaline phosphatase activity was measured in the media of transfected cells to control for variations in transfection efficiency between samples) were combined in a cationic lipid-DNA precipitate as per the manufacturer's instructions. Half of the precipitate was equally distributed over 3 wells in a 96-well plate, kept on the cells overnight, and replaced with fresh medium the following day. Forty-eight (48) hr after the start of the transfection, cells were treated and assayed for luciferase activity using a Luclite™ Kit (Packard, Cat. # 6016911) and "Trilux 1450 Microbeta" liquid scintillation and luminescence counter (Wallac) as per the manufacturer's instructions. The data were analyzed using GraphPad Prism™ 2.0a (GraphPad Software Inc.). Data are summarized below, where "CMV" is the control vector; "GPR35T" is the endogenous GPR35 receptor; and "GPR35(K216)" is the non-endogenous, constitutively activated version of the GPR35 receptor: